A PECULIAR SYSMEX SCATTERGRAM IN A CAT AFFECTED BY FELINE INJECTION SITE SARCOMA

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Signalment
A 13-year-old female, neutered, domestic short-haired cat.

History and clinical findings
The cat was presented to the Veterinary Teaching Hospital of the University of Milan for an interscapular mass already diagnosed as a mesenchimal malignant tumor by the referring veterinarian, based on cytological examination of the neoplasm.
At the presentation, the cat appeared in good shape and the clinical evaluation revealed only the presence of a mobile, solid, non painful and non ulcerated round mass, 1,5 X 1,4 cm in diameter, located in the subcutaneous tissue of the interscapular area.
The cat was regularly vaccinated and treated for ectoparasites with fipronil-based products. Tests for both feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) were never performed.

Diagnostic procedures and haematology
Cancer staging by total-body CT scan allowed to exclude the presence of loco-regional and distant metastasis. Wide margins excision of the neoplasm was performed, and the histological examination led to a definitive diagnosis of feline injection site sarcoma (FISS). A pre-surgical examination with wakeful cat was performed including biochemical analysis with automated spectrophotometer (Cobas Mira, Roche Diagnostics, Basel, Switzerland) and complete blood count (CBC) using the Sysmex XT-2000iV hematology laser analyzer (Sysmex Corporation, Kobe, Japan). A blood smear was also prepared and stained with a rapid staining (Hemacolor®, Merck, Damrstadt, Germany). Biochemistry was unremarkable except for mild hyperglycemia, most likely stress-induced (Table 1)

Table 1. Biochemical results (Cobas Mira, Roche Diagnostics, Basel, Switzerland) of the cat pre-surgical examination.

ANALYTE	DATA	REFERENCE INTERVAL
Urea (mg/dL)	54	(20-60)
Creatinine (mg/dL)	1.58	(<1.8)
Glucose (mg/dL)	**205**	(80-130)
Total Protein (g/dL)	7.13	(6.0-8.0)
ALT (U/L)	36	(<80)
ALP (U/L)	38	(<145)
Na^+ (mmol/L)	145	(145-156)
K^+ (mmol/L)	4.0	(3.4-4.8)
Cl^- (mmol/L)	118	(108-123)

Only a mild hyperglycemia is present.

Patient's erythrogram showed no significant alterations, while white blood cell count showed leukocytosis with slight neutrophilia and moderate eosinophilia.
The Sysmex WBC/DIFF scattergram showed an unusual cloud, located between the neutrophils, the eosinophils and the lymphocytes clouds and partially included in each of these populations gates. This cloud was clearly separated from the others when the scattergram was "switched" in the manual analysis frame (Figure 1)
On the day of surgery (24 days after the first blood sample), another blood sample was collected in order to repeat the CBC.
The Sysmex WBC/DIFF scattergram of the second blood sample was very similar to the first one, but the differential count was slightly different, since all the leukocyte parameters were within the reference intervals (Table 2).

Table 2. Summary of the cat haematological results at the first and at the second sampling obtained with Sysmex XT-2000iV analyzer.

ANALYTE	FIRST SAMPLE	SECOND SAMPLE	REFERENCE INTERVALS
RBC (x 10^{12}/L)	8.2	6.7	5,0-10,0
HGB (g/dL)	12.8	10.4	8,0-15,0
HCT (%)	38.4	31.2	24-45
MCV (fL)	46.8	46.5	39-55
MCH (pg)	15.6	15.5	14-19
MCHC (%)	33.3	32.1	31-35
PLT (10^9/L)	**72**	**32**	200-600
RDW	23.0	21.1	12-23
Total WBC (x 10^9/L)	**20,14**	13,81	6,0-17,0
Neutrophils (%)	66,0	47,4	35-75
Neutrophils (x 10^9/L)	**13,30**	6,55	2,5-12,5
Band (%)	-	-	< 3
Band (x 10^9/L)	-	-	< 0,3
Lymphocytes (%)	12,7	37,1	20-55
Lymphocytes (x10^9/L)	2,55	5,12	1,5-7,0
Monocytes (%)	3,3	2,4	1-4
Monocytes (x10^9/L)	0,66	0,33	0,0-0,85
Eosinophils (%)	18,0	13,1	2-12
Eosinophils (x10^9/L)	**3,63**	1,81	0,0-1,5
Basophils (%)	0	0	Rare
Basophils (x10^9/L)	0	0	Rare

First sample shows leukocytosis with neutrophilia and eosinophilia, while the second sample results are within the reference intervals.
Platelet count resulted adequated at the blood smear evaluation in both cases.

A double-blind manual differential count of the blood smear on two hundreds nucleated cells revealed a remarkable number of basophils (Figure 2), thus it could be hypothesized that the well separated cloud was represented by basophils.

Then, blood smear evaluation allowed to correct the differential leukocyte count, revealing the presence of basophilia in both samples (Table 3).

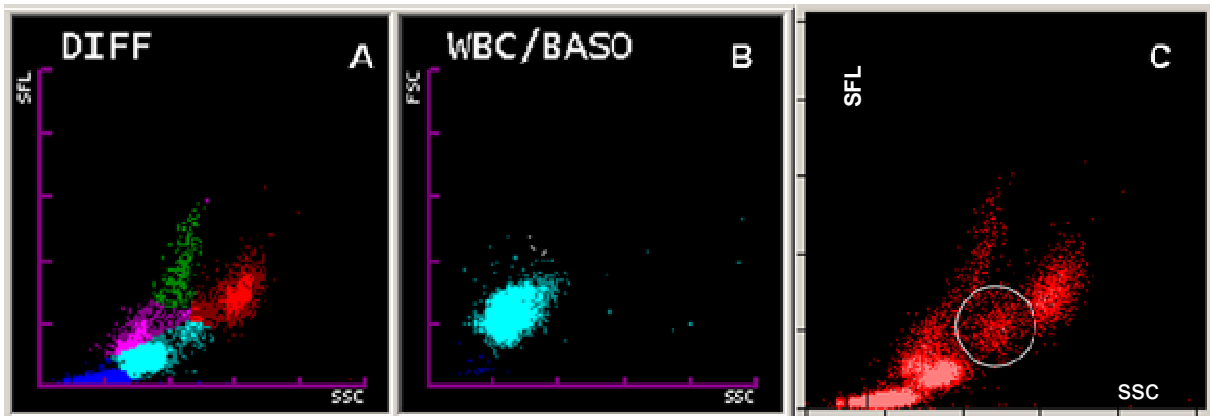

Figure 1
Sysmex XT-2000iV scattergrams of the feline blood leukocytes collected at the first sampling.
The WBC/DIFF scattergram (A) shows an additional cloud between the neutrophil, the eosinophil and the lymphocyte population. The separation of this "basophil" cloud is more evident when the scattergram is "switched" in the manual analysis frame (C, white circle). The WBC/BASO channel scattergram (B) shows no lysis-resistant population increases, reconfirming again that basophils are subjected to lysis as the other leukocyte population.
FSC: Forward scattered light; SFL: Side fluorescence light; SSC: Side scatter light.

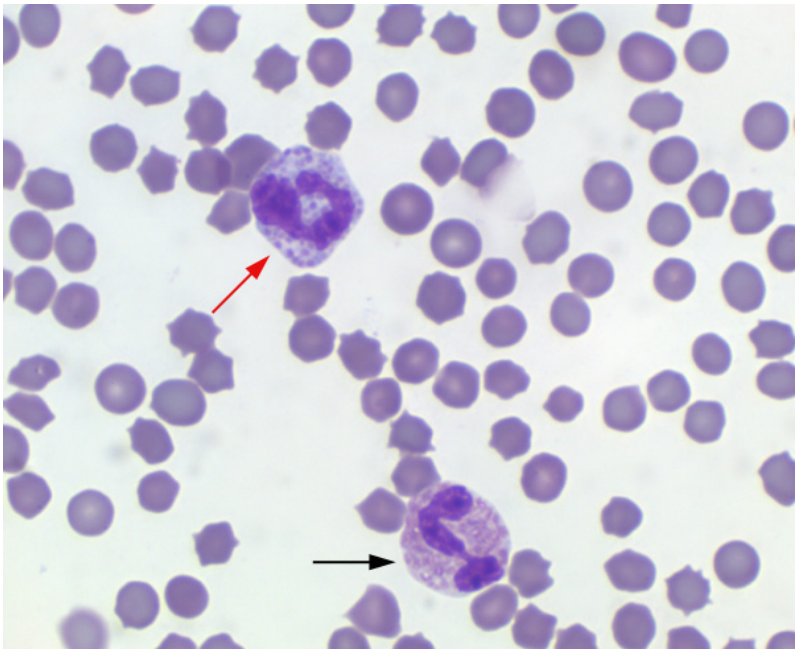

Figure 2. A basophil (red arrow) and an eosinophil (black arrow) in the blood smear from cat at the first sampling (Hemacolor®, Merck, Damrstadt, Germany).

Table 3: Sysmex XT 2000iV differential leukocyte count results compared with results obtained with manual count.

	First sample		Second sample		
Analyte	Sysmex	Manual	Sysmex	Manual	Reference intervals
Total WBC (x 10^9/L)	20.14	-	13.81	-	6.0-17.0
Neutrophils (x 10^9/L)	13.30	13.43	6.55	7.43	2.5-12.5
Band (x 10^9/L)	-	0.34	-	0.0	< 0.3
Lymphocytes (x10^9/L)	2.55	1.71	5.12	4.75	1.5-7.0
Monocytes (x10^9/L)	0.66	0.22	0.33	0.35	0.0-0.85
Eosinophils (x10^9/L)	3.63	3.52	1.81	0.94	0,0-1,5
Basophils (x10^9/L)	0.0	0.91	0.0	0.35	Rare

The first blood sample was collected for pre-surgery exams, while the second one was collected on the day of surgery. A manual differential count was performed and allowed to recognize basophilia in both cases.

Discussion

Basophils represent the least numerous granulocyte population in peripheral blood of cats, representing approximately the 0.5% of blood leukocytes in healthy cats [1]

Basophilia is intended as a massive or a persistent mild increase in blood basophils concentration. Since basophils are a minor leukocyte population and differential count, both manual and automated, suffers of imprecision, only increases above 200-300 basophils/µL should be defined as basophilia. [1,2]

This finding is rarely detected in domestic mammals and it is commonly associated with eosinophilia. The conditions responsible of basophilia are mostly IgE mediated disorders, such as allergic diseases and parasitism.[3] Several neoplastic diseases seem to be involved in paraneoplastic basophils increase, especially mast cell neoplasia with or without blood involvement. Basophilia in cats seems to be associated also with eosinophilic granuloma complex, basophilic leukemia, myeloid leukemia and polycitemia vera.[2]

Nevertheless, basophilia can be associated with other types of tumors, or it can rise due to not apparent reasons.[1,4]

Canine and feline basophils are not detected by most automated instruments, even if equipped with laser technology as Sysmex XT-2000iV.[5] Sysmex XT-2000iV performs the leukocyte count in two different channels: on the WBC/DIFF cytogram leukocytes are differentiated based on fluorescence and complexity. Specifically, cells are stained with a fluorescent polymethine agent after being permeabilized with a surfactant. Polymethine dye binds to nucleic acids and cytoplasmatic organelles, then the WBC differential count is realized through fluorescence flow cytometry using a red semiconductor laser at a wavelength of 633 nm.[6]

Cellular clusters are then separated with side fluorescence light (SFL) and laser side scatter light (SSC) and the results are displayed in a differential scattergram. Leukocytes with a high nucleic acids content, such as lymphocytes and monocytes, are found high in scattergram y-axis, which corresponds with the SFL. Neutrophils and eosinophils, because of their complexity, are found on the right of the x-axis, which corresponds to the SSC.[7]
In the DIFF channel, basophils are counted together with neutrophils, so the differential count considers the four major leukocyte population only.[6,8,9]
Basophils count is performed in the WBC/BASO channel, where the contact with a strong surfactant causes the loss of all leukocyte nuclei, except for human basophils.[5] Then the SSC and the forward scattered light (FSC) of lysed cells are determined and displayed on a second scattergram, with the SSC on the x-axis and the FSC on the y-axis.[6,10]
Unfortunately, it is well known that both in human and animal blood samples, automated analysers often fail in basophils identification.
A previous study about the occurrence and the enumeration of basophils in different species was performed comparing different haematology systems. Sysmex XT-2000iV failed to detect canine basophils, but the cytograms of several samples showed the presence of a basophil gate above the neutrophil population. This location is probably due to the cellular characteristics of basophils, showing a complexity similar to that of neutrophils, but a lower affinity for the fluorescent dye compared to eosinophils. Unfortunately feline blood samples were measured only with instruments different from Sysmex XT-2000iV.[5]
Recently, a report on the performances of the ProCyte Dx (IDEXX Laboratories, Westbrook, MA, U.S.A.), a haematological analyzer which combines both fluorescence laser flow cytometry and impedance and it is based on the same technology of Sysmex XT-2000iV, has been published[11].
Unlike Sysmex, the Procyte differential leukogram includes also the basophil population.[12]
Interestingly, in the ProCyte Dx feline dot plot (the analogous of the DIFF Sysmex-XT2000iv scattergram) basophils seem to be located in a position that is very similar to the basophil cloud that we found in the present case.
Nevertheless, in the study above mentioned, ProCyte Dx detected basophilia in 37 out of the 155 feline blood samples examined, while this finding was confirmed by the blood smear evaluation only in one case. The high number of false positive results suggests the need of further studies about feline basophils count with this cell counter.[11]
However, the similarity between published data on the Procyte analyser and the results obtained by us with the Sysmex-XT2000iV suggest that feline basophils have peculiar physico-chemical features that allow to identify a specific cloud in the Sysmex XT-2000iV scattergram.

Results of this case showed a cluster of cells presumably related with the basophil population that was shifted on the up-right side compared to the canine basophils clusters found in the study mentioned above.[5]
It could be hypothesized that this finding could be explained with the characteristics of feline basophils, in particular with their major cellular complexity compared to neutrophils and with the tendency to bind less fluorescent dye compared to eosinophils .
In conclusion, this case has two main interesting aspects: on one side, this case suggests that basophilia may be present in association with mesenchymal tumors, although the pathogenic mechanisms leading to basophilia cannot be defined in this study. On the other side, this report evidences that feline basophils may have physico-chemical properties that allow their identification in Sysmex scattergrams. Basophilia is reported as a rare occurrence, but the impossibility of basophils identification with haematology automated systems and their possible misidentification with other leukocytes on the manual count, may have caused the

underestimation of this leukocyte population in the past. However, the presence on the Sysmex XT-2000iV WBC/DIFF scattergram of an additional cloud as the one described in this case report should alert the operators to evaluate the blood smear, in order to provide a more accurate differential count.
Further studies are needed in order to verify the repeatability of these findings on a larger number of samples, especially in order to define the correlation between the size of the cloud and the rate of basophilia.

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